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(21) International Application Number: PCT/EP94/01498 (22) International Filing Date: 9 May 1994 (09.05.94) (30) Priority Data: 93201976.3 6 July 1993 (06.07.93) EP (34) Countries for which the regional or international application was filed: AT et al. (71) Applicant (for all designated States except US): QUEST INTERNATIONAL B.V. [NL/NL]; Huizerstraatweg 28, NL-1411 GP Naarden (NL). (72) Inventor; and (75) Inventor/Applicant (for US only): VAN DUJNHOFEN, Anton, A., G. [NL/NL]; Parallelweg 24, NL-4845 CR Wagenberg (NL). (74) Agent: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.
(54) Title: ENZYME CONTAINING PARTICLES (57) Abstract The invention relates to enzymes which are in a particulate, non-dusting form and which are substantially non-allergic. The particles according to the invention comprise a binding agent which is substantially insoluble in water and having dispersed therein an enzyme, which enzyme is released upon the application of shear forces. Prior to the application of shear forces, the particles according to the invention do not exhibit substantial enzymatic activity.		

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ENZYME CONTAINING PARTICLES

The invention relates to particles containing enzymes which enzymes are released in a controllable way after shear has
5 been applied to the particles.

Enzymes are widely applied in the foods industry. Such enzymes are often produced by microorganisms (e.g. fungi, yeast or bacteria) in a fermenter. Generally, at some stage
10 of the production process the microorganisms are separated from the culture medium, whereafter the aqueous solution containing enzymes is dried by e.g. freeze drying or spray drying to yield a dry enzyme powder. This is a traditional way in which food grade enzymes are produced and offered.
15 This dry form leads to at least two major problems: dusting and allergic reactions.

The dusting problem is due to the very fine powder which is usually obtained by drying enzyme solutions or dispersions.
20 Dusting can lead to e.g. spoilage of (expensive) material, contamination, difficult dosing of the enzyme, etcetera. In short: dusting causes difficulties in controlling the enzymes, which is highly undesirable in modern processing.

25 Various solutions to the dusting problem have been proposed in the past. Such a solution is for example sticking or gluing enzyme particles to coarser non-enzymatic particles to form particles which are considerably larger than the individual enzyme particles which thus reduces the dusting
30 tendency. Such a solution for enzymes for use in detergents is disclosed in e.g. the British Patent nr. 1344253 and US patent 3,801,463. Spray-drying of enzymes, optionally together with suspenders and thickeners and salts is also disclosed in US patent 4,233,405.

35

Although the formation of large, non-dusting particles containing enzymes is a workable solution to prevent

dusting, the disadvantage of causing allergic reactions can only be overcome by a controlled release of the enzyme at the spot where enzyme activity is desired, while the enzyme should not be released when it is not desired.

5

There is a growing use of enzymes in food processing industry. In many cases enzymes used in food processing need to be active in the stage right after they are added to a food composition comprising a substrate for the enzymes. Additionally, the enzymes should preferably be in a non-dusting form.

In US patent 4,310,554 the purpose is to protect and maintain the activity of enzymes in a favourable microenvironment by incorporating them in microcapsules. The microcapsules may serve as a vehicle to incorporate curing agents (including enzymes) into cheese. The microcapsules are prepared in situ in the milk which is used for making cheese. The shell of the microcapsules is milkfat. The aqueous content of the microcapsules is released during cheese making by a heat treatment. This will cause the microcapsule wall to melt.

In US patent 3,561,975 it is described that the shrinkage of pie crust dough during baking can be lowered by adding a protease to the dough which is not active during the pre-baking stage, but becomes active upon baking the dough. This is achieved by incorporating dry protease particles in shortening in such a way that large shortening particles are produced having embedded therein in discrete regions solid protease particles. It is stated that the coating (shortening) shields the enzyme up to the moment when sufficient heat is applied (during baking) causing the shortening to melt, whereafter the protease becomes active in breaking down a part of the gluten, which, when not broken down causes shrinkage of the pie crust dough. It is stated that it is not desired to have the protease

liberated before dough baking, because this would lead to an excessive weakening of the dough due to premature breakdown of the gluten network.

5 The last two patents discussed above show methods for a controlled delivery of enzymatic activity in foodstuffs for two very specific applications. The method as disclosed in US patent 4,310,554 has the disadvantage that the microcapsules are obtained in an aqueous medium, thus not
10 yielding dry, easy to store particles. The method described in US patent 3,561,975 also relates to a specific purpose: release of a protease during the baking stage in the preparation of pies. Furthermore, it is not reported that the protease containing particles according to US patent
15 3,561,975 do not cause allergenic reactions. The process for making particles according to US patent 3,561,975 also requires that a large number of parameters are carefully controlled, e.g. the enzyme particle size and shape in relation to the size and shape of the prepared particle
20 containing the enzyme particle. Both cited references have in common that they disclose the release of enzymes upon the application of heat.

In view of these limited solutions to the problems as set
25 out earlier, there is a need for enzymes for use in foodstuffs which are in a particulate, non-dusting form, which are easy and safe to store and process, which are non-allergic and easy to obtain, while the enzymes are released or become active during mixing at least a part of
30 the ingredients (or shortly thereafter) to which such enzyme containing particles are added.

It has now been found that these objectives can be met by preparing and using particles comprising a binding agent,
35 which binding agent is substantially insoluble in water and dispersed in the binding agent an enzyme, which enzyme is released upon the application of shear forces. Prior to the

application of shear forces, the particles according to the invention do not exhibit substantial enzymatic activity. This means that after preparation of the particles according to the invention the enzymes are shielded from the environment by immobilising them with said binding agent until shear is applied where and when the enzyme activity is desired.

Since the binding agent is insoluble in water when solid, the enzyme containing particles according to the invention are not prone to give allergic reactions. This is due to the fact that when coming into contact with e.g. mucosal tissue of the human body, the enzyme containing particles according to the invention will not dissolve or otherwise disintegrate and thus the enzyme (which may lead to allergic reactions) will not be released.

The fact that the enzymes are liberated by applying shear forces makes the enzyme containing particulate material according to the invention distinctly different from the enzyme containing particles so far, since in these known cases the enzymes are liberated due to melting of the material which is combined with the enzymes.

The shear forces needed to release the enzyme can be provided by any means, e.g. by conventional food mixing or kneading means when processing the mixture to which the enzyme particles according to the invention are applied. Thus there is no need for any additional equipment involved in processing the foodstuffs, since mixing is applied in almost any process of preparing foodstuffs.

The particles according to the invention are also characterized in that the enzyme is substantially homogeneously distributed in the particles. This is a feature which makes the particles according to the invention distinguishable from the enzyme containing

particles known so far, in which the enzymes are present in either one discrete region (e.g. in microcapsules) or in a number of discrete regions (e.g. small enzyme particles glued together or to a carrier material).

5

It is already stated that a convenient binding agent for the particles according to the invention is a compound or composition which is substantially insoluble in water when solid. It is preferred, however, that when this binding agent is in its molten or liquid form, it is well miscible with water or an aqueous solution or dispersion. Particular suitable as the binding agent are compounds or compositions which, when in liquid or molten state, easily form a physical or chemical interaction with water molecules when added thereto. Examples of such compounds or compositions are emulsifiers. A preferred binding agent for the particles according to the invention comprises at least 50% of an emulsifier, and even more preferred is a binding agent which comprises at least 80% of an emulsifier. Since the major interest for applying enzyme particles according to the invention is in the food industry, food grade emulsifiers are preferred for this purpose. Most preferred emulsifiers for this purpose are emulsifiers which comprise a mono- or diglyceride. Preferred binding agents have melting points between 30°C and 90°C, more preferably between 50°C and 70°C.

The particles according to the invention may be applied to a plastic mixture, which may be a food mixture. In such a plastic mixture the enzyme is released by applying shear forces to the mixture. Among such plastic mixtures are food compositions, food components or food raw materials which are processed in some way. A preferred way of applying shear forces to the plastic mixture comprising the particles according to the invention is (or involves) kneading or extruding the mixture.

In a preferred embodiment of the invention the enzyme particles according to the invention are (a part of) so-called baking improvers which are added to dough or a dough ingredient to improve the qualities of the dough and/or of the baked goods prepared thereof. Enzymes may be used e.g. to increase loaf volume of bread, reduce staling or improve dough tolerance. A common feature of these enzymes is that they need to be active prior to baking, thus during kneading or right thereafter, e.g. during a possible fermenting, rising or proofing stage of the dough. The particles according to the invention are very suitable for addition to dough or dough ingredients. This has the advantage that no extra processing step or processing means are needed, since doughs are always thoroughly kneaded. A baking improver may comprise other components besides enzymes which may have a beneficial effect on the properties of dough or of the baked goods prepared thereof. Thus baked goods prepared by baking a plastic mixture as set out above form another embodiment of the invention.

20

Such baking or dough improving enzymes which may be added in the form of particles according to the invention may comprise one or more carbohydrate modifying enzymes and/or one or more oxidoreductases and/or one or more proteases.

25

Preferred carbohydrate modifying enzymes are α -amylase, xylanase, β -mannanase or mixtures thereof. Preferred oxidoreductases are glucose-oxidase, sulfhydryl-oxidase, SS-isomerase, SS-transferase or mixtures thereof.

30

To limit dusting of the enzyme containing particles according to the invention, it is preferred that at least 90 % of the particles according to the invention has a size of at least 50 μ m, and preferably of at least 100 μ m. If the particles become much smaller, dusting may still occur.

The particles according to the invention can be prepared in

a number of ways. Preferably, the particles according to the invention comprising an enzyme and a binding agent are prepared by subsequent:

- 5 a) mixing an aqueous solution or dispersion of the enzyme with the molten binding agent,
- b) particulation of the obtained mixture,
- c) cooling the obtained particulates to solidification.

10 Preferably, the particulation as disclosed above under step b) is achieved by using a nozzle. Cooling as mentioned under step c) of the process as set out above is preferably achieved by using a spray cooling tower. More preferably, spray cooling is combined with particulation by a nozzle.

15 Although in the preceding description enzyme containing particles are described, the disclosed method of shielding a food ingredient component from its environment may be applied to other ingredients than enzymes. This is in particular applicable to other ingredients which are
20 applied to foodstuffs in minor amounts, like colorants, flavours, antioxidants etcetera. It is also possible to combine various food ingredients in the particles according to the invention. In the case of bakery applications,
25 enzymes together with other baking improvers like salts and/or yeast may be incorporated together in the particles according to the invention.

In the examples, the amount of α -amylase added to dough is
30 expressed in SKB units. These units are well defined.

Measurement of the amylase activity:

α -Amylase activity is determined according to the modified SKB method described by Olson, Evans & Dickson
35 (Cereal Chem. 21, 533, 1944). The principle is that starch forms a coloured complex with iodine, while α -amylase degraded starch (dextrins) does not.

The invention is exemplified by the following examples but is in no way limited thereto.

EXAMPLE 1: Enzyme activity

- 5 In this example it is measured whether particles according to the invention exhibit enzymatic activity in wet conditions, at ambient temperature and without substantial shear. An enzyme mixture comprising xylanase and α -amylase was used. The activities in water of both the enzyme
10 mixture as such as well of enzyme containing particles according to the invention were measured. The following materials were tested:

Prepared:

- | | |
|-----------------------------|---------------------------------|
| 15 enzyme mixture as such | 80.000 units xylanase |
| (flour as carrier material) | 500 SKB units α -amylase |
| particles according to the | 80.000 units xylanase |
| invention containing: | 500 SKB units α -amylase |
| 20 (1 g particles) | |

Enzyme activity measured by immersion in water (100 ml, 20°C) after mixing enzymes with water, activity calculated on dry weight:

25

Measured:

enzyme mixture as such	80.000 units xylanase
	500 SKB units α -amylase

- 30 No detectable enzyme activity could be measured when the particles according to the invention were immersed in water.

- It can be concluded from this example that the particles
35 according to the invention do not exhibit enzymatic activity in wet conditions at ambient temperatures in the absence of shear.

EXAMPLE 2: baking performance

The performance of enzyme particles according to the invention when applied to dough is measured by preparing
5 baked goods according to the following process:

	<u>Raw Materials</u>	<u>Gram</u>
	Flour T550 *	2000
10	Water	1200
	Baker's Yeast	60
	Salt	40
	Ascorbic acid	0.1
	Fat	20
15	* manufacturer Wessanen, The Netherlands	
Processing:		
	Kneading machine	: "Kemper Spiral"
20	Kneading time	: 2 min. low speed, 3 minutes high speed
	Dough temperature	: 26°C
	Dough weight	: 400 g
	"To round up"	: by Hand
25	First proofing time	: 25 minutes
	Moulder	: "Mono" moulder
	Second proofing time	: 25 minutes
	Final moulder	: "Mono" moulder
	Final proofing	: 70 minutes, 32°C, 80% humidity
30	Baking	: 30 minutes at 240°C

In this experiment a reference loaf of bread was baked according to the above recipe (reference).

Tests were performed with the following additives per kg of flour:

1. reference: no additives
- 2 conventional enzyme mix: 80.000 units xylanase *
500 SKB units α -amylase **
3. mono/diglyceride mix: 0.2 gram Admul MG 6203 **
4. particles according to the invention comprising:
80.000 units xylanase
500 SKB units α -amylase
0.2 gram mono/diglyceride ***
5. conventional enzyme mix and mono/diglyceride:
80.000 units xylanase
500 SKB units α -amylase
0.2 gram mono/diglyceride.

* Dutch patent application 90.01388 example 1.5.6

** Manufacturer Quest International the Netherlands

*** all mono/diglyceride used as the binding agent in the particles according to the invention.

The loaves of bread obtained by baking the above 5 doughs were stored enclosed in plastic in a temperature controlled cabinet at 25°C. The results are shown in table 1. It can be concluded from table 1 that enzyme containing particles according to the invention are substantially equal in baking performance compared to conventional enzyme mixes (example no. 4 compared to no. 2 and/or no. 5). From this it can be concluded that the enzymes incorporated in the particles according to the invention as in test 4 are substantially completely liberated upon kneading and/or fermentation. Mono- and diglycerides are not active as improver at the applied dosage levels (example no. 2

Table 1: baking results for loaves.

No.	Enzyme/monodi applied in baking	monodi glyceride (g/Kg flour)	Activity Xylanase α -amylase	(units/Kg flour)	Freshness (1=best) in hours (4=worst)		
					24	48	72 96
1.	Reference	-	-	-	4/5	4/5	4/5 4/5
2.	Conventional enzyme mix	-	80.000	500	1/3	1/3	1/3 1/3
3.	Mono/diglyceride	0.2	-	-	4/5	4/5	4/5 4/5
4.	Enzyme particles according to the invention	0.2	80.000	500	1/3	1/3	1/3 1/3
5.	mono/diglyceride and conventional enzyme mix	0.2	80.000	500	1/3	1/3	1/3 1/3

compared to no. 5), but may of course be active at higher dosing levels.

EXAMPLE 3: baking performance: specific volume and dough consistency

5 In this example crispy rolls are used.

The effects on specific volume and dough consistency (and stability) are more pronounced/critical in this application than with loaves of bread. The following formulation and
10 processing have been used to make the crispy rolls:

Recipe:

	<u>Gram</u>
15 Flour (T550)	1000
Water	600
Baker's yeast	40
Salt	20
Ascorbic acid	0.05

20

Processing:

	Kneading machine	: Kemper Spiral
	Kneading time	: 2 min. at low speed
25		3 min. at high speed
	Dough temperature	: 26°C
	Bakery temperature	: 23-25°C
	First proofing time	: 15 min. to round up
	Second proofing time	: 15 min. to divide and round up
30	Rest proofing time	: 3 min. to shape German rolls
	Final proofing time	: 50 or 65 minutes

Tests were done with per kg flour the following additives:

35 6. reference: no additives

7. conventional enzyme mix: 20.000 units xylanase *

500 SKB units α -amylase **

8. mono/diglyceride mix: 0.2 gram Admul MG 6203 **

5 9. particles according to the invention comprising:

20.000 units xylanase

500 SKB units α -amylase

0.2 gram mono/diglyceride ***

10 10. conventional enzyme mix and mono/diglyceride:

20.000 units xylanase

500 SKB units α -amylase

0.2 gram mono/diglyceride.

15

The experiment has been carried out in such a way that Reference was taken as a reference and the tests with the improvers were carried out in duplicate or triplicate. After the initial kneading, 30 rolls were made and

20 individually assessed on the following characteristics:

1. Dough consistency during processing

1 = dry/stiff dough

4 = flexible dough

25

2. Dough stability during transport to the oven (after final proofing time)

-- unstable dough

++ stable dough

30

3. Specific volume (SV) in % according to the rapeseed replacement method.

4. Form (shape of the rolls)

35

1 = very bad, no split, flat roll

10 = very good, open split, round roll

The results are shown in Table 2 below:

Table 2

5 The effect of encapsulated enzymes on dough and
 form of crispy rolls

10	No.	Dough		Form		SV in %	
		consist- ency	stabi- lity	proofing 50'	time 65'	proofing 50'	time 65'
	6.	1	-	5	3	100	100
	7.	3	+	8	7	108	109
	8.	1	-	5	4	100	101
15	9.	3	+	8	7	108	109
	10.	3	+	8	7	109	109

From table 2 it is clear that enzyme containing particles
20 according to the invention are equal in baking performance
compared to conventional enzyme mixes. Mono/diglycerides are
not active as improver at these dosage levels.

CLAIMS

1. Particles comprising a binding agent which is substantially insoluble in water and which particles have dispersed in the binding agent an enzyme, which enzyme is released upon the application of shear forces.
2. Particles comprising a binding agent which is substantially insoluble in water and which particles have dispersed in the binding agent an enzyme, which enzyme is substantially homogeneously dispersed in the binding agent.
3. Particles according to claim 1-2, characterized in that the binding agent is soluble in or miscible with water when the binding agent is molten.
4. Particles according to claim 1-3, characterized in that the binding agent comprises at least 50% of an emulsifier.
5. Particles according to claim 4, characterized in that the binding agent comprises at least 80% of an emulsifier.
6. Particles according to claim 4-5, characterized in that the emulsifier is a food grade emulsifier.
7. Particles according to claim 4-6, characterized in that the emulsifier comprises a mono- or diglyceride.
8. Particles according to claim 1-7, characterized in that the enzyme comprises a carbohydrate modifying enzyme.
9. Particles according to claim 1-7, characterized in that the enzyme comprises an oxidoreductase.

10. Particles according to claim 8, characterized in that the enzyme comprises α -amylase, xylanase, β -mannanase or a mixture thereof.
- 5 11. Particles according to claim 9, characterized in that the oxidoreductase comprises glucose-oxidase, sulfhydryl-oxidase, SS-isomerase, SS-transferase or a mixture thereof.
- 10 12. Particles according to any one of claims 1-11, characterized in that 90 % of the particles has a diameter of at least 50 μ m, preferably of at least 100 μ m.
- 15 13. A plastic mixture comprising particles according to claim 1-12.
14. A plastic mixture comprising particles according to claim 1-12, wherein the enzyme is released by applying
20 shear forces to the mixture.
15. A plastic mixture according to claim 14, characterized in that the shear forces are caused by kneading or extruding the mixture.
25
16. A plastic mixture according to any one of claims 13-15, characterized in that the plastic mixture is dough.
17. Baking improver comprising particles according to claim
30 1-12.
18. Baked goods prepared by baking a plastic mixture according to claim 13-16.
- 35 19. Process for producing particles comprising an enzyme and a binding agent, characterized in that the particles are obtained by subsequent:

- a) mixing an aqueous solution or dispersion of the enzyme with the molten binding agent,
b) particulation of the obtained mixture,
c) cooling the obtained particulates to solidification
5 of the binding agent.
20. Process according to claim 19, characterized in that the particulation is achieved by using a nozzle.
- 10 21. Process according to claim 19 or 20, characterized in that particulation and cooling are achieved by using a spray cooling tower.

INTERNATIONAL SEARCH REPORT

International Application No

PC 94/01498

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A21D8/04 C12N9/96

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A21D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A,2 072 988 (COLGATE-PALMOLIVE COMPANY) 24 September 1971 see page 1, line 40 - page 7, line 14 ---	1-15, 19-21
X	EP,A,0 256 127 (SHOWA DENKO KABUSHIKI KAISHA) 24 February 1988 see the whole document ---	1-5,8-15
X	US,A,3 527 644 (LANDFRIED, B.W.) 8 September 1970 see the whole document ---	1-21
X	EP,A,0 246 554 (RÖHM GMBH) 25 November 1987 see the whole document ---	1-21
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,3 561 975 (LUEBERING, J.E.) 9 February 1971 cited in the application see the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

inform on patent family members

International Application No

PCT/JP 94/01498

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